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A new species of *Helminthosporium* from Jiangsu, China

Xiao-Jing Wang, Hai-Yan Wu, & Meng Zhang*

Henan Agricultural University Zhengzhou, Henan, China, 450002
* Correspondence to: *zm2006@126.com*

Abstract— *Helminthosporium nanjingense* sp. nov. is described from Jiangsu Province, China, on dead branches of an unidentified tree. The new fungus produces a conspicuous yellow-green pigment on PDA media. Its microscopic features and ITS sequence are provided.

Key words — systematics, saprobes, hyphomycetes

Introduction

During the recent survey of *Helminthosporium* species in China, the authors discovered eleven new species and seven new records (Zhang & Zhang 2009, Zhang et al. 2003, 2004, 2007, 2010). In this paper we propose a new species of *Helminthosporium* from Jiangsu Province, China.

Materials & methods

Unidentified dead branches with *Helminthosporium* species were carefully inspected under a Nikon SMZ1500 stereomicroscope. Tissues mounted in a drop of lactophenol were examined microscopically. Conidia and conidiophores were photographed using a Nikon Eclipse 80i microscope with Nikon DS-U2 camera, and microscopic measurements were completed by using NIS-Elements Image Analysis Software.

DNA was obtained from mycelia scraped from single spore cultures of the strains on a PDA plate and extracted using the Cell Genomic DNA Isolation Kit (LifeFeng, Shanghai, China) following the manufacturer's instructions. A final volume of 25 µL [comprising 1 µL ITS1 forward sequencing primer (5µM) and 1 µL ITS4 reverse sequencing primer (5 µM), 9.5 µL ddH_2O, 12.5 µL 2× Taq PCR Master Mix (including 0.1 U/µL Taq DNA Polymerase, 0.4 mM each dNTPs, 2× Taq Buffer, red and blue indicator dye, PCR enhance reagent and proteide steady reagent), 1 µL fungal DNA] was amplified using a Eppendorf PCR thermal cycler device (Germany) as follows: 95°C initial denaturation (2 min), 33cycles of 95°C (30 s), 55°C (30 s), 72°C (2 min), and a final step of 72°C (7 min). The 5-µL PCR products were analyzed through electrophoresis on a 1.0% agarose gel with 1× Tris-acetate-EDTA (TAE electrophoresis buffer). The PCR products were sequenced by Shanghai Sangon Biological Engineering Technology & Service Co. Ltd., China (Xie et al. 2010; Schoch et al. 2012). The type specimen is deposited in HSAUP (Herbarium of Shandong Agricultural University: Plant Pathology), and the ex-type culture in HHAUF (Herbarium of Henan Agricultural University: Fungi).

Taxonomy

Helminthosporium nanjingense Meng Zhang, Xiao J. Wang & H.Y. Wu, **sp. nov.**

FIG. 1

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Differs from other similar species of *Helminthosporium* by its smooth-walled, longer, slightly thinner conidia and its longer conidiophores.

TYPE: China, Jiangsu Province, Nanjing, on dead branches of an unidentified tree, 8 Sep. 2002, coll. M. Zhang (**Holotype**, HSAUP$_{02}$0198; ex-type culture, HHAUF$_{02}$0380=ZM$_{02}$0380; GenBank KF192322).

ETYMOLOGY: Latin, *nanjingense,* for the collection locality.

COLONY on the natural substrate effuse, black, hairy. Mycelium mostly immersed in the substratum. COLONY on PDA media effuse with yellow-green pigment. CONIDIOPHORES arising solitary or in fascicles from the cells of the mycelium, simple, straight or flexuous, thick-walled, sub-cylindrical, smooth, brown to dark brown, 250–470 µm long, 6.9–7.7 µm wide, with well-defined small pores at the apex and laterally beneath the upper 1–4 septa; 8.6–11 µm wide at the base cell which sometimes inflated up to 15.5 µm. CONIDIA subulate or nearly whip-like, straight or curved, thin-walled, smooth, pale brown, 6–17 distoseptate, 64.5–170.5 µm long, 7.3–10.3 µm wide in the widest part, narrowing towards the apex to 5.0–6.8 µm wide, with a blackish-brown scar at the base, 1.4–2.7 µm thick.

COMMENTS: *Helminthosporium nanjingense* is most closely related to *H. conidiophorellum* Meng Zhang & T.Y. Zhang and *H. subhyalinum* Meng Zhang & T.Y. Zhang in conidial morphology (subulate or nearly whip-like shape). *Helminthosporium conidiophorellum* differs from *H. nanjingense* by its shorter and slightly wider conidia (100–147.5 × 9.5–11 µm) that are sometimes slightly verruculose and its shorter wider conidiophores (60–280 × 7.0–8.5 µm) (Zhang & Zhang 2009). *Helminthosporium subhyalinum* is differentiated by its nearly colorless, shorter, slightly wider conidia (72–125 × 9.5–11.5 µm) and shorter conidiophores (120–200 µm); the thick black conidial apex wall of *H. subhyalinum* is also a striking distinguishing characteristic (Zhang et al. 2007).

The amplified rDNA (ITS) region derived from the holotype was 454 bp (GenBank KF192322). The BLAST comparison with previously published *H. solani* and *H. velutinum* sequences shows only 89% nucleotide homologies for both. Reference sequences for comparative analysis were obtained from

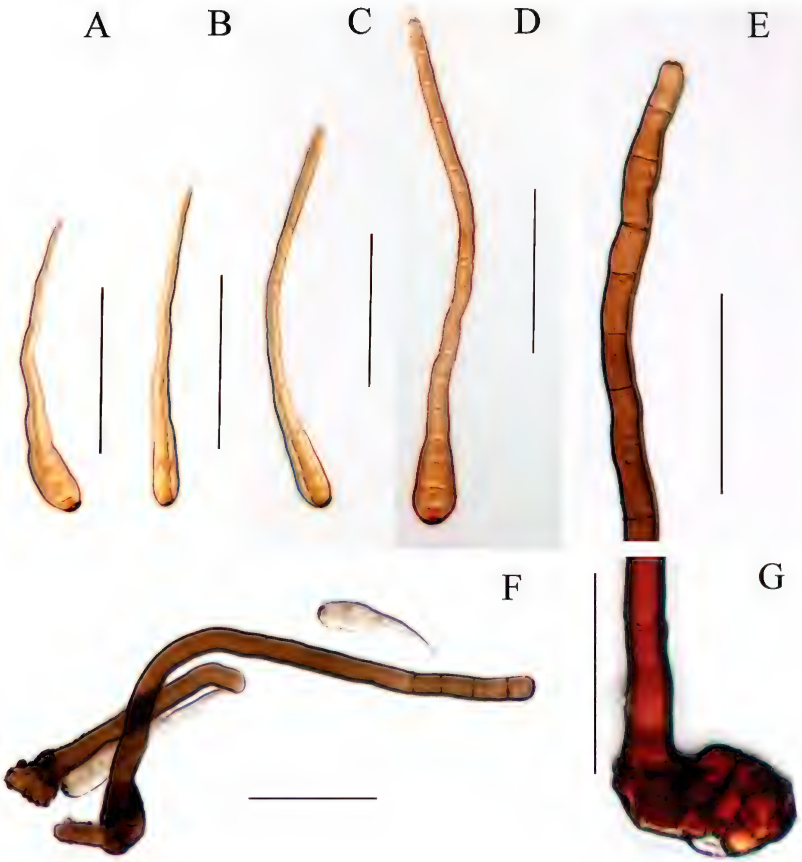

Fig. 1 *Helminthosporium nanjingense* (holotype HSAUP $_{02}$01980): A–D. Conidia; E. Conidiophore apex; F. Conidiophores; G. Conidiophore base. Scale bars = 50 µm.

GenBank database by the BLAST search program (http://www.ncbi.nlm.nih.gov/BLAST/).

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